Autophagy Is Activated in Colorectal Cancer Cells and Contributes to the Tolerance to Nutrient Deprivation

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Abstract

Several types of cancer cells, including colorectal cancer–derived cell lines, show austerity, the resistance to nutrient starvation, but exactly how cancer cells obtain energy sources under conditions in which their external nutrient supply is extremely limited remains to be clarified. Because autophagy is a catabolic process by which cells supply amino acids from self-digested organelles, cancer cells are likely to use autophagy to obtain amino acids as alternative energy sources. Amino acid deprivation-induced autophagy was assessed in DLD-1 and other colorectal cancer–derived cell lines. The autophagosome-incorporated LC3-II protein level increased after treatment with a combination of autolysosome inhibitors, which interferes with the consumption of autophagosomes. Autophagosome formation was also morphologically confirmed using ectopically expressed green fluorescent protein-LC3 fusion proteins in DLD-1 and SW480 cells. These data suggest that autophagosomes were actively produced and promptly consumed in colorectal cancer cells under nutrient starvation. Autolysosome inhibitors and 3-methyl adenine, which suppresses autophagosome formation, remarkably enhanced apoptosis under amino acid–deprived and glucose-deprived condition. Similar results were obtained in the cells with decreased ATG7 level by the RNA interference. These data suggest that autophagy is pivotal for the survival of cancer cells. Furthermore, some aggressive malignant tumors, such as pancreatic cancers, are clinically hypovascular (4). Under these conditions, cancer cells are likely to encounter a shortage of nutrients. These cells might use some alternative metabolic process to obtain energy for their survival. We have reported that several cancer cell lines, including pancreatic cancer–derived and colorectal cancer–derived cell lines, are resistant to the nutrient-deprived culture condition. For example, colon cancer–derived SW480 and DLD-1 cells showed >50% survival after 36 h of culture in a glucose-deprived, amino acid–deprived, and serum-deprived medium. On the other hand, untransformed human fibroblasts and liver cancer–derived cells were completely abolished under the same condition. We named this starvation-resistant phenotype “austerity” and speculated that this phenomenon may contribute to the survival of cancer cells in nutrient-deficient microenvironment (5, 6).

It remains to be elucidated how cancer cells obtain energy sources under the condition in which the supply of external nutrients is extremely limited. Mammalian cells are able to use amino acids as an alternative energy source. If the starvation-resistant cancer cells can raise amino acids from the inside of cells, the amino acids become potential energy sources under such condition.

Autophagy is a conserved catabolic process by which cells self-digest their organelles (7). At the first step of autophagy, a lipid bilayer structure called the isolation membrane is developed. Isolation membrane sequesters cytoplasmic materials, such as organelles, to form autophagosomes. During this step, LC3, one of the mammalian homologues of yeast ATG8, is processed and activated by an ubiquitination-like reaction regulated by ATG7 and ATG3 (8). First, LC3 proform is cleaved into a soluble form known as LC3-I. LC3-I is further modified into a membrane-bound form, LC3-II, and recruited onto the autophagosomes. Autophagosomes engulfing organelles then fuse with lysosomes and mature into autolysosomes. Sequestrated materials are digested to amino acids in the autolysosomes by the lysosomal enzymes. In this step, LC3 and other autophagosome components are also digested and diminished. Several pharmacologic autophagy inhibitors have been used to evaluate the physiologic relevance of autophagy in culture cells. The combination of E64d and pepstatin A inhibits lysosomal enzymes and interferes with autolysosomal digestion, whereas 3-methyladenine (3-MA) blocks autophagosome formation to inhibit autophagy (9).

Autophagy has roles in protecting cells against shortage of nutrients. Cells supply amino acids from self-digested organelles as an alternative energy source for their survival (7). This function of autophagy seems ideal to foster cancer cells to survive in an unfavorable starved microenvironment. However, the physiologic

Introduction

Proliferating cancer cells require nutrients for growth. Tumor angiogenesis is one way to increase the blood flow to supply the required oxygen and energy source to the growing tumor tissues. However, recent studies have revealed that oxygen and glucose levels are frequently reduced in locally advanced tumors even after tumor vessels have been established (1–3). It suggests that the tumor microvasculature is structurally and functionally abnormal and not sufficient to supply the blood flow needed by proliferating cancer cells. Furthermore, some aggressive malignant tumors, such as pancreatic cancers, are clinically hypovascular (4). Under these conditions, cancer cells are likely to encounter a shortage of nutrients. These cells might use some alternative metabolic process to obtain energy for their survival. We have reported that several cancer cell lines, including pancreatic cancer–derived and colorectal cancer–derived cell lines, are resistant to the nutrient-deprived culture condition. For example, colon cancer–derived SW480 and DLD-1 cells showed >50% survival after 36 h of culture in a glucose-deprived, amino acid–deprived, and serum-deprived medium. On the other hand, untransformed human fibroblasts and liver cancer–derived cells were completely abolished under the same condition. We named this starvation-resistant phenotype “austerity” and speculated that this phenomenon may contribute to the survival of cancer cells in nutrient-deficient microenvironment (5, 6).

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*Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).*

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relevance of autophagy in tumor formation and progression is still controversial. We hypothesized that autophagy contributes to the acquisition of austerity in cancer cells and investigated whether autophagic machinery is activated in colorectal cancer cells and tissues. We also examined whether the inhibition of autophagy induced death of colorectal cancer cells, which had acquired austerity.

**Materials and Methods**

**Cells and culture.** Human colorectal cancer cell lines SW480, DLD-1, WiDr, SW620, and LoVo were purchased from the American Type Culture Collection. Cells were maintained in DMEM (Invitrogen) supplemented with 10% FCS, 4.5 g/L glucose, and antibiotics. Amino acid starvation was done with an amino acid–deprived medium as described previously (6). E64d, pepstatin A, and 3-methyl adenine (3-MA) were purchased from Sigma.

**Patients and tissue samples.** Subjects comprised 65 patients with 80 adenocarcinomas, who underwent surgical removal of colorectal cancer in the National Cancer Center Hospital East (Kashiwa, Japan) from 2004 to 2006. Twenty tubular adenomas and 13 hyperplastic polyps, which were endoscopically resected, were also collected. All patients agreed to enrollment in the study and each gave informed consent. The institutional review board of the National Cancer Center approved all protocols on the patients' agreement. In the present study, only tumors that have penetrated through muscularis mucosae into submucosa are considered adenocarcinoma according to the WHO classification. Tubular adenomas were also classified into low grade or high grade, depending on the degree of glandular complexity, extent of nuclear stratification, and severity of abnormal nuclear morphology (WHO classification).

**Western blotting.** Production of rabbit polyclonal LC3 and ATG7 antibodies was described previously (8, 10). Phosphorylated Akt (Ser473) and phosphorylated AMPKα1 (Ser 485) antibodies were purchased from Cell Signaling Technology. Cellular protein was extracted from culture cells and tissue specimens using lysis buffer [1% SDS, 10 mmol/L Tris-HCl (pH 7.4), 1 mmol/L Na3VO4], and protein concentration was determined with a bicinchoninic acid protein assay kit (Pierce). Ten micrograms of total cell lysates were separated by 15% Tris-glycine SDS-PAGE and then transferred to a polyvinylidene difluoride membrane. Membranes were blocked with TBS containing 5% (w/v) dry milk and 2% (w/v) bovine serum albumin with 0.1% Tween 20. After washing in TBS, membranes were incubated for 1 h at room temperature with horseradish peroxidase–conjugated goat anti-rabbit IgG antibody (Santa Cruz Biotechnology) in 1:10,000. Signals were detected using enhanced chemiluminescence detection reagents (GE Healthcare). The density of each band was measured using ImageJ software (W.S. Rasband, NIH, Bethesda, MD).

References:

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Establishment of green fluorescent protein-LC3–expressing cells and detection of the fusion protein. A green fluorescent protein (GFP)-mouse LC3 fusion protein expressing vector pEGFP C2-LC3 was kindly provided by Dr. Hitoshi Okada (Ontario Cancer Institute, Toronto, Ontario, Canada). SW480 and DLD-1 cells (5 × 10⁴ per well) were plated on six-well plates the day before transfection and 0.25 μg of plasmid DNA was transfected using TransFast Transfection Reagent (Promega) according to the manufacturer’s protocol. Transfected cells were selected by 1 mg/mL of G418 sulfate (potency: 803 μg/mg; Calbiochem) for 14 days. GFP fusion proteins were observed using an LSM5 PASCAL laser scanning microscope system (Carl Zeiss Japan). Autophagosome-incorporated fusion proteins were measured using ImageJ software.

RNA interference. ATG7 RNA interference was accomplished by transfecting DLD-1 cells with the specific small interfering RNA (siRNA). ATG7-targeting siRNA and nonsilencing siRNA were purchased from Dharmacon. Short oligo-RNAs were transfected using DharmaFECT 1 transfection reagent (Dharmacon) as recommended by the manufacturer. Cells were cultured for 48 h before analysis.

Cell death assay. Cell viability was assessed by the Hoechst 33342 (Sigma) staining as described previously (11). Treated cells were stained and examined under fluorescent microscopy. At least 1,000 cells were counted and distinguished as viable and apoptotic cells.

Immunohistochemistry. Tissue sections were deparaffinized and exposed to 3% hydrogen peroxide for 15 min to block endogenous peroxidase activity. For heat-induced epitope retrieval, the sections were placed in a 0.01 mol/L citrate buffer and heated at 120°C for 15 min. The nonspecific binding was blocked by preincubation with 2% normal swine serum in PBS (blocking buffer). The slides were then incubated overnight at 4°C with an anti-LC3 antibody at a final concentration of 2 μg/mL in the blocking buffer. The slides were washed with PBS and then incubated with a peroxidase-labeled polymer conjugated to goat anti-rabbit IgG (DAKO EnVision Peroxidase Rabbit) for 45 min at room temperature. After extensive washing with PBS, the color reaction was developed in 2% 3,3′-diaminobenzidine in 50 mmol/L Tris buffer (pH 7.6) containing 0.3% hydrogen peroxide for 5 to 10 min. The sections were then counterstained with Mayer’s hematoxylin, dehydrated, and mounted.

Transmission electron microscopy. Surgically resected tissue samples were fixed in ice-cold 2% glutaraldehyde and examined with a JEM-1011 transmission electron microscope (JEOL).

Statistical analysis. Data were analyzed using the Student’s t test for statistical significance. P values were considered significant if <0.05. SD was calculated and represented in the bar graphs.

Results

Amino acid deprivation induced LC3-II accumulation in colorectal cancer cells. First, we assessed the induction of autophagy in colorectal cancer–derived culture cells. DLD-1 cells were cultured for 24 h in amino acid–deprived medium with or without a combination of lysosomal protease inhibitors E64d and pepstatin A. Amino acid deprivation induced increased phosphorylation of AMPKα in DLD-1 cells. Autolysosome inhibitors slightly enhanced the phosphorylation level of AMPKα (Fig. 1A). On the other hand, LC3-II level was slightly induced by amino acid deprivation, but addition of autolysosome inhibitors dramatically enhanced LC3-II level in a dose-dependent manner (Fig. 1A). Treatment with autolysosome inhibitors enhanced the accumulation of LC3-II in other colorectal cancer–derived cell lines, WiDr, SW620, and DLD-1 cells (Fig. 1B). The LC3-II level of SW480 and LoVo cells in amino acid–deprived medium without autolysosome inhibitors was less than that in amino acid–containing medium. However, the autolysosome inhibitors significantly increased LC3-II levels of these cells too (Fig. 1B).

Autophagosome formation was visualized using GFP-LC3 fusion protein. Then, we confirmed autophagosome formation in live cells. We established SW480 and DLD-1 cells that stably expressed the GFP-LC3 fusion protein. These cells were cultured in amino acid–deprived medium with or without the combination of E64d and pepstatin A for 12 h. The distribution of GFP-LC3 was determined by confocal fluorescence microscopy, and autophagosome-associated GFP-LC3 level was quantified (Fig. 2A and B). The GFP-LC3 fusion protein was observed as coarse dots in the cytoplasmin both SW480 and DLD-1 cells cultured in an amino acid–deprived medium. Consistent with the Western blotting data,
autophagosome inhibitor treatment enhanced the dot formation. In contrast, 3-MA, which inhibits autophagosome formation, significantly reduced the visible dots in the treated cells and redistributed GFP-LC3 to the cytoplasm. Taking these findings together, autophagosomes were properly produced, processed, and consumed in these colon cancer–derived cell lines under amino acid starvation.

**Inhibition of autophagy induced apoptosis of colorectal cancer cell lines during nutrient starvation.** To examine the effect of autophagy in tumor cell survival during nutrient starvation, the autophagy inhibitors were applied to examine the cell viability of SW480 and DLD-1 cells in amino acid–deprived medium. Hoechst 33342 staining showed limited death of both cell lines during 48-h culture in amino acid–deprived medium (Figs. 3 and 4A). On the contrary, addition of autolysosome inhibitors showed marked chromatin condensation, indicating apoptotic cell death (Fig. 3). This effect was dependent on the time of the treatment and dose of the inhibitors (Fig. 4A). Increased apoptosis was confirmed by flow cytometry with Annexin V and propidium iodide double staining (data not shown). 3-MA treatment also showed marked chromatin condensation during amino acid starvation (Figs. 3 and 4B). Autolysosome inhibitors and 3-MA also induced apoptosis of SW620 cells in which functional autophagosome formation was confirmed (Figs. 1B and 4B). Similar results were obtained in the cells treated with ATG7-targeting siRNA. DLD-1 cells with decreased ATG7 expression showed increased apoptosis in amino acid–deprived medium (Fig. 4C). E64d and pepstatin A treatment enhanced apoptosis of DLD-1 in amino acid–deprived and glucose-deprived medium. However, these inhibitors did not affect the cell viability in nutrient-containing medium (Fig. 4D). These results strongly suggest that the autophagy has a protective role against nutrient deprivation-induced death of austeric colorectal cancer cells.

**LC3-II is highly expressed in colorectal cancer tissues but not in normal epithelia.** Because tumor cells are frequently exposed to nutrient starvation in vivo, we speculated that the protective role of autophagy may play some roles in tumor cell survival during tumorigenesis. We evaluated autophagy in surgically resected colorectal cancer tissues by detecting LC3. Paraaffin-embedded samples of 65 cases with 80 adenocarcinoma lesions were examined by immunohistochemistry using an anti-LC3 antibody. Immunohistochemical expression for LC3 in tumor and normal epithelia was estimated by comparing the staining intensity in the submucosal or muscularis nerve plexus cells that consistently express LC3 (Fig. 5A). Tumor or nontumorous mucosal epithelia showing equal or stronger intensity than nerve cells were judged as "strongly positive," whereas the epithelia with weaker intensity than nerve cells were designated as "weakly positive." LC3 was not detected in the normal mucosa of all 65 patients. In contrast, we found positive staining for LC3 in the most of primary cancer tissues. In 80 adenocarcinomas, 59 were strongly positive and 13 were weakly positive. There was no significant difference of the LC3 positivity between well-differentiated and moderately differentiated adenocarcinomas (Fig. 5A; Table 1). Close examination of LC3-positive cells showed that the LC3 protein localized in the cytoplasm with irregular condensation (Fig. 5A). This distribution was similar to that of GFP-LC3 recruited onto autophagosomes in cultured colorectal cancer cells (Fig. 2). The LC3 status of metastatic lesions was also examined. Five metastatic adenocarcinomas in liver were all LC3 positive (data not shown). Six metastatic adenocarcinomas in liver were also LC3 positive (Supplementary Figure). To confirm whether LC3 deposition reflects autophagosome formation, an LC3-positive adenocarcinoma specimen was examined with transmission electron microscopy. Cancer cells contained lipid bilayer structures engulfing organelles in the cytoplasm, which are characteristic for autophagosomes (Fig. 5B). To evaluate which type of LC3 was produced in cancer tissues, total cell lysates were prepared from five patients with surgically resected colorectal adenocarcinoma and LC3 was detected by Western blotting. A metastatic liver adenocarcinoma specimen and a noncancerous liver tissue specimen were also prepared from one patient. Autophagosome-incorporated LC3-II was a dominant form of LC3 in tumor tissues. Consistent with the immunohistochemical staining, the LC3-II level in four of five primary adenocarcinoma samples and in a metastatic adenocarcinoma sample in liver was higher than that in each corresponding noncancerous tissue, including liver (Fig. 5C). These results suggest that the autophagic machinery is activated in colorectal cancer tissues.

**LC3 accumulation in benign tumors.** We next examined LC3 positivity in benign tumors. LC3 was not accumulated in 13 hyperplastic polyps as well as the nontumorous mucosal epithelium. Among 10 high-grade tubular adenomas, 4 were negative, 5 were weakly positive, and 1 was strongly positive for LC3, whereas 10 low-grade tubular adenomas were negative for LC3 as well as nontumorous mucosal epithelium and hyperplastic polyps (Fig. 5D; Table 1).
Discussion

The role of autophagy in the cell fate decision remains controversial. Autophagy is claimed to play roles in degradation of old cellular components, recycling constituents and responding to various cellular stresses especially those of energy deficiency (7). Recently, autophagy has attracted much attention in the connection with programmed cell death, autophagic death. Autophagic death eliminates damaged and/or harmful cells, such as cancer cells treated with anticancer reagents or the cells infected with pathogenic microorganisms (12, 13). On the other hand, autophagy is also claimed to be an indispensable physiologic reaction to sustain cell viability under nutrient-starved conditions (7).

The controversy about the physiologic roles of autophagy is also argued for cancer development and biology. In an experimental pancreas cancer model, increased autophagy was observed in the step of the tumor formation (14). Starvation-induced protein degradation was not down-regulated among transformed and transformed-tumorigenic bronchial epithelial cells compared with their normal counterparts (15). A high potential of autophagic protein degradation was observed in an undifferentiated colon cancer cell line HT29 (16). On the contrary, the basal rate of degradation of long-lived proteins in hepatoma cells was less than that of their noncancerous counterparts under normal nutrient condition (17, 18). Recently, several tumor-related gene products have been identified as autophagy regulators. The hemiallelic loss of \( \text{BECN} \), a mammalian homologue of yeast \( \text{Atg6} \), led to tumor formation in mammary glands, ovaries, and prostates (19). \( \text{BECN} \) coding protein, Beclin1, was later focused on as stimulating autophagy and suppressing tumorigenesis (20). It is suggested that mammalian target of rapamycin, which is regarded as a mediator of oncogenic signals, is involved in the negative control of autophagy and suppressing tumorogenesis (21). The \( \text{TP53} \) tumor suppressor gene product trans-activates \( \text{DRAM} \), which encodes a lysosomal protein that induces autophagy and is involved in p53-mediated cell death (22).
contradictory findings suggest that there is not a unique paradigm about autophagy in tumorigenesis.

We have proposed that austerity is an important determinant of the malignancy of cancer (5, 6). Cancer cells with austerity can survive under conditions in which the exogenous nutrient supply is extremely limited. Under these conditions, cancer cells require alternative energy sources, such as amino acids. Autophagy is likely to be a way to supply amino acids for these cells.

In this study, we showed that colorectal cancer cells harbor functional autophagosomes. The membrane-bound LC3-II protein level is regarded as a marker for the formation of autophagosomes (9, 23). We detected LC3-II with Western blotting using anti-LC3 antibody (Fig. 1). The autophagosome level is dynamically regulated in the cells undergoing autophagy. Under nutrient starvation, autophagosomes are rapidly degraded by lysosomal enzymes. The LC3-II level indicates the balance between the production and the consumption of autophagosomes. When the consumption is highly increased, the LC3-II level seems unchanged or even decreases comparing with that of non-autophagic cells. Interference of the degradation processes preserves LC3-II from consumption, and the accumulated LC3-II level reflects the amount of newly produced autophagosomes. In fact, amino acid deprivation without inhibitors slightly increased the LC3-II level in DLD-1 and SW620 cells, whereas there was no significant induction of LC3-II in WiDr, LoVo, and SW480 cells. However, treatment with autolysosome inhibitors markedly increased LC3-II in all tested cell lines. These data suggest that the autophagic machinery was functionally activated in a series of cultured colorectal cancer cells. Accumulation of autophagosomes was also visualized (Fig. 2). It has been reported that the GFP-fused LC3 protein was recruited to autophagosomes and degraded in autolysosomes as native LC3 proteins (23, 24). Recently, it was reported that transiently expressed fusion protein caused artificial accumulation in nonautophagic cells (25). To avoid the artifact, we prepared SW480 and DLD-1 cells stably expressing the GFP-LC3 protein. These cells showed characteristic dot formation in amino acid–deprived medium, suggesting that the fusion proteins were successfully recruited to the autophagosomes. Inhibition of autophagosome consumption by autolysosome inhibitors increased the dot formation of GFP-LC3 in SW480 and DLD-1 cells. In contrast, 3-MA, which interferes with autophagosome formation, apparently decreased the dots.

We showed that autophagy directly contributes to the survival of cancer cells under nutrient starvation. Both the autolysosome inhibitors and 3-MA induced marked apoptotic death of all examined colorectal cancer cells (Figs. 3 and 4). Interestingly, the concentration of inhibitors that induced cell death in nutrient-deprived medium did not affect the viability of the cells in the amino acid–containing medium. We observed that 3-MA successfully reduced the autophagosome formation in GFP-LC3–expressing SW480 cells in the amino acid–containing and glucose-containing medium (data not shown). This suggests that cancer cells may not depend on autophagy when sufficient nutrients are available, but suppression of autophagy is fatal in a starved environment. It was reported that inhibition of autolysosome function sensitized HeLa cells to apoptosis (26). Our observation was consistent with their findings. Furthermore, we suggest that autophagy has a pivotal role in acquiring the tolerance to amino acid deprivation in the cancer cells with austerity as well as the starvation-sensitive HeLa cells.

It has been well described that AMPK-mediated signaling pathway senses the intracellular energy status and regulates metabolism to maintain cellular homeostasis. We have reported that AMPK and its related kinases are key molecules to obtain austerity (27–29). Recently, it was reported that AMPK positively regulates autophagy (30). Activation of AMPK by amino acid

Figure 5. LC3-II was expressed in colorectal cancer tissues. A, LC3 protein was detected with immunohistochemistry using paraffin-embedded colon cancer samples. Top left, HE staining. Original magnification, ×100. Top right, LC3 immunostaining. Original magnification, ×100. LC3 was detected in cancer tissue (C) and intermuscular nerve cells (Nv). Bottom, magnified LC3-positive cancer tissue. Original magnification, ×400. B, ultrastructure of a surgically resected colon cancer shown by electron microscopy. Lipid bilayer structures engulfing organelles (*) are shown in the cytoplasm. Nucl, nucleus. Original magnification, ×2,000. C, total cell lysates were prepared from surgically resected primary and metastatic liver adenocarcinoma specimens and LC3-II was detected with Western blotting. N, noncancerous tissue; M, metastatic liver adenocarcinoma tissue; NL, noncancerous liver tissue. Case 1 had two independent primary tumors. D, LC3 was not detected in nonneoplastic tubules (N), whereas low-grade adenomatous component (L) showed weak LC3 positivity and high-grade adenomatous component (H) showed strong LC3 positivity. Original magnification, ×100.
We observed that glucose starvation also induces AMPK phosphorylation. Harris AL. Hypoxia—a key regulatory factor in tumour cell deprivation was observed in DLD-1 cells in this study (Fig. 1A). We observed that glucose starvation also induces AMPK phosphorylation in DLD-1 cells (data not shown), suggesting that depletion of amino acids and/or glucose induces decreased energy sources of cancer cells. Autolysosome inhibitors canceled the starvation-resistant phenotype in nutrient-deprived medium (Fig. 4). These findings strengthen the idea that autophagy provides intrinsic alternative energy sources and contributes to the survival of cancer cells under nutrient starvation.

Evaluating autophagy in clinical tumor samples has been difficult mainly due to the lack of appropriate autophagy-specific markers. In the present work, we showed that the LC3 immunohistochemical staining is a useful surrogate marker for autophagy in surgically resected cancer specimens. The LC3 protein was accumulated specifically in adenocarcinoma cells but not in surrounding nontumorous mucosal epithelial cells (Fig. 5). Accumulated LC3 protein was supposed to be involved in the autophagosome formation. Subcellular distribution of LC3 was similar to that of autophagosome-incorporated LC3 in culture cells. The majority of the LC3 protein was a membrane-bound LC3-II seen in Western blotting. In addition, the ultrastructure revealed the presence of autophagosomes in the cytoplasm.

Although LC3 accumulation showed a distinct contrast between adjacent nontumorous and tumor epithelial cells, the nutrient supply is assumed to be similar between these tissues. It suggests that the autophagy in colorectal cancer cells is regulated not only by the extracellular nutrients concentration but that some intrinsic mechanisms are also involved in the regulation. Aberrantly proliferating cancer cells require extra energy sources than normal cells. It is likely that the cells with active autophagy have an advantage for survival, especially when the cells do not have alternative ways to obtain extra energy sources, such as tumor angiogenesis.

If autophagy supplements energy sources in cancer cells, autophagosome formation might be increased as the nutrient deprivation was extended. Degenhardt et al. reported an experimental cancer model using transplanted transformed mouse kidney epithelial cells to nude mice. In their study, autophagosome formation was enhanced preferentially at the center of the tumor mass, which was distant from the vasculature and supposed to be more starved (31). In this study, we detected accumulated autophagosomes in some high-grade tubular adenomas as well as advanced cancers and metastatic lesions. We speculate that cancer cells in vivo require autophagy in early stages of tumorigenesis. Once the tumor cells are selected, these cells might maintain the active autophagy. We also assumed that colorectal cancer cells are typical cell types that acquired the higher basal activity of autophagy. We observed that the all tested colorectal cancer–derived cell lines produced comparatively high level of LC3-II protein without induction of autophagy (Fig. 1). The LC3-II protein could not be detected in HeLa cells under the same condition (data not shown).

Taken together, we conclude that active autophagy contributes to tumor cell survival in colorectal cancers in vitro and in vivo. We also speculate that the pathophysiologic roles of autophagy in tumorigenesis may vary among each type of cancer due to the different biological characteristics of cancer cells and their microenvironments. We would like to extend our investigation to other cancers and clarify the specific regulatory mechanism of autophagy and its relevance to cancer formation and progression.

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References


Table 1. LC3 expression in colorectal cancer tissues

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